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NOTES

Outer Membrane Penetration by (2,3)-Methylenepenams

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The penetration of the *Escherichia coli* outer membrane by two sterically restricted analogs of penicillin G was determined. The analog corresponding to the "open" conformation of penicillin G penetrated faster than the "closed"-form analog did, and both analogs penetrated faster than penicillin G did. The results suggest that the conformation of the β-lactam nucleus may affect penetrability via the porin-mediated pathway.

The ability of antibiotics to penetrate the bacterial outer membrane rapidly is an important determinant of their antibacterial activity. Antibiotics which penetrate poorly may not reach concentrations sufficient to inactivate their targets, particularly if they themselves are inactivated in transit by bacterial enzymes (8, 9, 17).

β-Lactam antibiotics diffuse through the outer membrane mainly via the OmpF and OmpC porins in *Escherichia coli* (4, 10). For a given β-lactam antibiotic the rate of diffusion is affected by molecular weight, ionic charge, and hydrophobicity. Diffusion through the major porins is limited to compounds with a mass of approximately 600 daltons or less (11). Decreased hydrophobicity correlates well with increased penetrability (20), and cationic and dipolar ionic compounds diffuse through the outer membrane faster than anionic ones do (12). Steric factors also seem to affect outer membrane penetration in some cases (19). Outer membrane permeability is further complicated by the presence of nonporin pathways, their relative importance being dependent on the particular compound (3, 13).

The availability of sterically restricted analogs of penicillin G allowed examination of the effect of penam conformation on penetrability. The penam nucleus can exist in either the "open" or the "closed" form; the open penam has been postulated to be the biologically active isomer (5). The (2,3)-α- and (2,3)-β-methylenepenams shown in Fig. 1 are rigid analogs of the open and closed conformations, respectively, of penicillin G. The isomers differ from penicillin G in that they have one of the methyl groups at C-2 as part of a cyclopropyl structure. In addition, the α-isomer has a reduced carboxylic acid–β-lactam torsional angle. The two isomers had been previously examined for the effects of their structures on chemical reactivities and biological activities (2, 5; C.-C. Wei, K.-C. Luk, K. F. West, J. L. Roberts, D. L. Pruess, P. Rossman, M. Weigele, and D. D. Keith, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1292, 1986; D. D. Keith, J. Christenson, N. Georgopapadakou, V. Madison, D. Pruess, P. Rossman, and L. Todaro, 26th ICAAC, abstr. no. 1293, 1986). The α-isomer had antibacterial activity comparable to that of penicillin G against both the wild type (UB1005) and a permeability mutant (DC2) (15) of *E. coli*, while the β-isomer was inactive (2).

In the present study, the outer membrane penetrability of both isomers was assessed by the method of Zimmerman and Rosselet (20) with *E. coli* RC709 (7), a strain containing the R1 plasmid, which encodes a TEM-1 β-lactamase (6). The permeability coefficient was calculated from Fick's law of diffusion as described by Nikaido and Vaara (13). β-Lactamase-producing progeny of *E. coli* JF701 (*ompC264*) and JF703 (*ompF254*) (10) were constructed by conjugation with *E. coli* RC709 and selection by ampicillin resistance and resistance to phage SS4 (OmpC specific) or phage K20 (OmpF specific). JF701 and JF703 were generously provided by J. Foulds, and the phages were a gift from C. Schnaitman. Penicillin G was obtained from Eli Lilly & Co., and penicillin V was obtained from Sigma Chemical Co. The (2,3)-methylenepenams were synthesized in the Department of Anti-Infective Chemistry, Hoffmann-La Roche Inc. Hydrolysis of the β-lactams was determined by the iodometric method of Sargent (16). The hydrophobicity of the antibiotics was determined by the method of Tsuiji et al. (18).

The kinetic parameters of the TEM-1 β-lactamase for the β-lactams, necessary for calculating the permeability coefficient were determined from Lineweaver-Burk plots. The TEM-1 enzyme had a decreased affinity for the two (2,3)-methylenepenam isomers and lower physiological efficiency (14) when compared with penicillin G (Table 1), although the kinetic parameters were still within the range typical for penicillins (1). The K_ms for penicillin G and penicillin V,

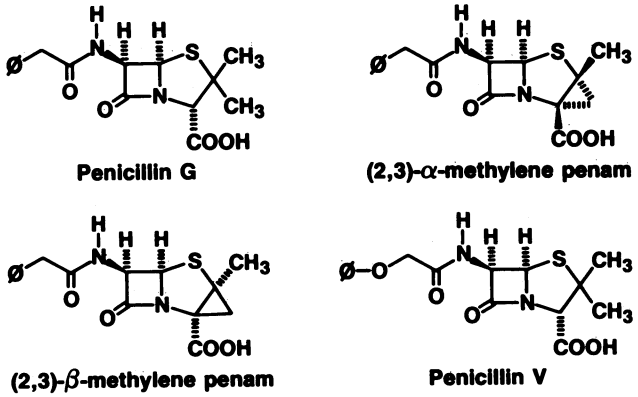

FIG. 1. Structures of compounds used in this study.

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TABLE 1. Kinetic parameters of TEM-1 β-lactamase for penicillin G and related compounds[a]

Compound	K_m (μM)	V_{max} (nmol/min per mg [dry wt])	Physiological efficiency[b] (10^3)
Penicillin G	29 ± 6.2	264 ± 13.2	9.1 ± 0.14
(2,3)-α-Methylenepenam	70 ± 3.9	104 ± 12.7	1.5 ± 0.18
(2,3)-β-Methylenepenam	76 ± 4.8	231 ± 9.9	3.0 ± 0.7
Penicillin V	32 ± 4.6	306 ± 9.6	9.6 ± 0.13

[a] Values are the means ± standard errors for four to six determinations with sonicated extracts of *E. coli* RC709.
[b] V_{max}/K_m (8).

included as reference values, were similar to those described previously (20).

The (2,3)-α- and (2,3)-β-methylenepenam isomers penetrated the outer membrane of wild-type *E. coli* with permeability coefficients nearly five times and two times that of penicillin G (Table 2). The periplasmic concentrations attained by the α- and β-isomers were, respectively, 27- and 4-fold higher than those attained by penicillin G. Penicillin V, a penam structurally related to penicillin G but more hydrophobic, penetrated at a lower rate and attained periplasmic concentrations lower than those attained by penicillin G.

Penetrability through the OmpF and OmpC porins was examined separately to determine the relative contribution of each porin to increased penetrability. The two (2,3)-methylenepenam isomers diffused faster through both the OmpF and OmpC porins than penicillin G did (Table 3). All three compounds diffused through OmpF faster than OmpC, the α-isomer having a larger OmpF/OmpC permeability coefficient ratio than penicillin G or the β-isomer (3.0 versus 1.2 and 1.3, respectively). The α-isomer diffused as well as the β-isomer through OmpC but three times as well as the β-isomer through OmpF.

The hydrophobicity of the compounds was examined to determine if it was responsible for the observed differences in penetrability. The P_u values obtained were as follows: penicillin G, 1.66 ± 0.06; (2,3)-α-methylenepenam, 1.78 ± 0.08, (2,3)-β-methylenepenam, 1.76 ± 0.14; and penicillin V, 1.95 ± 0.07. The values obtained for penicillin G and penicillin V agree with those described previously (18).

The α-isomer had antibacterial activity comparable to that of penicillin G against both the wild type (UB1005) and a permeability mutant (DC2) (15) of *E. coli*, while the β-isomer was inactive (2).

The twofold-higher penetrability of the α-isomer relative to the β-isomer through OmpF (Table 3) suggests a role for the conformation of the penam nucleus in determining penetrability. The flatter shape of the α-isomer and the larger diameter of the OmpF porin (120% of the area of OmpC) may

TABLE 2. Penetration of (2,3)-methylenepenams into wild-type *E. coli*[a]

Compound	Periplasmic concn (μM)	Permeability coefficient (cm/s) (10^7)
Penicillin G	0.82 ± 0.099	0.41 ± 0.05
(2,3)-α-Methylenepenam	22.2 ± 4.6	1.93 ± 0.3
(2,3)-β-Methylenepenam	3.45 ± 0.32	0.77 ± 0.07
Penicillin V	0.41 ± 0.09	0.29 ± 0.04

[a] The external antibiotic concentration was 1.0 mM. Values are the means ± standard errors for at least three experiments.

TABLE 3. Penetration of (2,3)-methylenepenams through OmpF and OmpC porins[a]

Strain[b]	Compound	Periplasmic concn (μM)	Permeability coefficient (cm/s) (10^7)
JF701 R^+ ($OmpF^+$)	Penicillin G	0.63 ± 0.14	0.42 ± 0.09
	(2,3)-α-Methylene-penam	55.3 ± 14.9	3.46 ± 0.6
	(2,3)-β-Methylene-penam	8.26 ± 0.82	1.73 ± 0.16
JF703 R^+ ($OmpC^+$)	Penicillin G	0.48 ± 0.06	0.32 ± 0.05
	(2,3)-α-Methylene-penam	10.8 ± 3.1	1.20 ± 0.3
	(2,3)-β-Methylene-penam	8.4 ± 1.0	1.43 ± 0.39

[a] The extracellular penam concentration was 1.0 mM. Values are the means ± standard errors for at least three experiments.
[b] R^+ indicates that the strains were β-lactamase-producing strains constructed by conjugation with *E. coli* RC709.

allow denser packing of the α-isomer molecules as they move through the OmpF porin channel. Such packing of molecules may not be possible within the smaller OmpC porin. The β-isomer is incapable of such an arrangement.

Several β-lactams described in the literature have a relationship similar to that of (2,3)-α-methylenepenam and penicillin G. Cephalexin and cefaclor are cephem analogs of ampicillin (19). They differ from ampicillin in that they have a β-lactam nucleus that resembles the open form of the penam nucleus, an unsubstituted C-2 atom, smaller carboxylic acid–β-lactam torsional angles, and hydrophobic substituents at C-3. Despite their increased mass and hydrophobicity relative to those of ampicillin, both cephalexin and cefaclor penetrate the outer membrane of *E. coli* approximately three times faster than ampicillin does (19). Generally, increased hydrophobicity of monoanionic β-lactams correlates with decreased penetrability through the outer membrane (19, 20). However, in the examples presented above, increased penetrability was achieved despite increased hydrophobicity. Thus, the conformation of the β-lactam nucleus may be an important determinant of penetrability, and steric factors may sometimes overshadow hydrophobicity in determining penetrability.

The higher periplasmic concentration attained by the (2,3)-α-methylenepenam isomer relative to penicillin G was not reflected in its antibacterial activity (2). This result was most likely due to the decreased affinity of the α-isomer for all *E. coli* penicillin-binding proteins except PBP 3 (2) coupled with the chemical instability of the α-isomer (half life, 6.5 h at pH 7.0). The present study points to the limitations of the use of MICs for wild-type strains and their permeability mutants in making conclusions about penetrability. Some compounds, like (2,3)-β-methylenepenam, may simply lack antibacterial activity; others, like (2,3)-α-methylenepenam, may have altered properties that affect penetrability and intrinsic activity differently.

LITERATURE CITED

1. **Fisher, J. F., and J. R. Knowles.** 1978. Bacterial resistance to β-lactams: the β-lactamases. Annu. Rep. Med. Chem. **13**:239–248.
2. **Georgopapadakou, N. H., D. A. Russo, A. Liebman, W. Burger, P. Rossman, and D. D. Keith.** 1987. Interaction of (2,3)-methylenepenams with penicillin-binding proteins. Antimicrob. Agents Chemother. **31**:1069–1074.

3. **Hancock, R. E. W.** 1984. Alterations in outer membrane permeability. Annu. Rev. Microbiol. **38**:237–264.
4. **Jaffe, A., Y. A. Chabbert, and O. Semonin.** 1982. Role of porin proteins OmpF and OmpC in the permeation of β-lactams. Antimicrob. Agents Chemother. **22**:942–948.
5. **Keith, D. D., J. Tengi, P. Rossman, L. Todaro, and M. Weigele.** 1983. A comparison of the antibacterial and β-lactamase inhibiting properties of penam and (2,3)-β-methylene penam antibiotics. Tetrahedron **39**:2445–2458.
6. **Lindqvist, R. C., and K. Nordström.** 1970. Resistance of *Escherichia coli* to penicillins. VII. Purification and characterization of a penicillinase mediated by the R factor R1. J. Bacteriol. **101**:232–239.
7. **Meynell, E., and R. C. Datta.** 1967. Mutant drug resistant factors of high transmissibility. Nature (London) **214**:885–887.
8. **Murray, B. E., and R. C. Moellering.** 1978. Patterns and mechanisms of antibiotic resistance. Med. Clin. N. Am. **62**:899–923.
9. **Neu, H. C.** 1984. Changing mechanisms of drug resistance. Am. J. Med. **77**:11–23.
10. **Nikaido, H.** 1985. Role of permeability barrier in resistance to beta-lactam antibiotics. Pharmacol. Ther. **27**:197–231.
11. **Nikaido, H., and E. Y. Rosenberg.** 1981. Effect of solute size on diffusion rates through the transmembrane pores of the outer membrane of *E. coli*. J. Gen. Physiol. **77**:121–125.
12. **Nikaido, H., E. Y. Rosenberg, and J. Foulds.** 1983. Porin channels in *Escherichia coli*: studies with β-lactams in intact cells. J. Bacteriol. **153**:232–240.
13. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of outer membrane permeability. Microbiol. Rev. **49**:1–32.
14. **Pollock, M. R.** 1965. Purification and properties of penicillinases from *Bacillus licheniformis*: a chemical, physicochemical, and physiological comparison. Biochem. J. **94**:666–675.
15. **Richmond, M. H., D. C. Clark, and S. Wotton.** 1976. Indirect method for assessing the penetration of beta-lactamase-nonsusceptible penicillins and cephalosporins in *Escherichia coli* strains. Antimicrob. Agents Chemother. **10**:215–218.
16. **Sargent, M. G.** 1968. Rapid fixed-time assay for penicillinase. J. Bacteriol. **95**:1493–1494.
17. **Smith, J. T.** 1983. Mechanisms of drug resistance, p. 79–83. *In* D. J. Edwards and D. R. Hiscock (ed.), Chemotherapeutic strategy. Macmillan Publishing Co., New York.
18. **Tsuiji, A., O. Kubo, E. Miyamoto, and T. Yamana.** 1977. Physicochemical properties of β-lactam antibiotics: oil-water distribution. J. Pharm. Sci. **66**:1675–1679.
19. **Yoshimura, F., and H. Nikaido.** 1985. Diffusion of β-lactam antibiotics through porin channels of *Escherichia coli* K-12. Antimicrob. Agents Chemother. **27**:84–92.
20. **Zimmerman, W., and A. Rosselet.** 1977. Function of the outer membrane of *Escherichia coli* as a permeability barrier to beta-lactam antibiotics. Antimicrob. Agents Chemother. **12**: 368–372.